Quantitative structure–activity studies of octopaminergic ligands against *Locusta migratoria* and *Periplaneta americana*

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Abstract: The quantitative structure–activity relationship (QSAR) of octopaminergic agonists and antagonists against the thoracic nerve cord of the migratory locust, *Locusta migratoria*, was analysed using physicochemical parameters and regression analysis. Sixty-five molecules that employ a single [^{3}H]octopamine (OA) binding criterion were selected. The hydrophobic and electronic nature of the ligands were the most important factors for binding activity: the positive log of octanol-water partition coefficient (Log *P*), dipole moment (*DM*) and negative valence connectivity index (*CVI1*) terms mean that the more hydrophobic, the larger the *DM* value and the smaller the *VCI1* value, the greater the binding activity. The quantitative structure–activity relationship of 2-(arylimino)oxazolidines (AIOs), 2-(aralkylamino)-2-oxazolines (AAOs), 2-(arylimino)thiazolidines (AITs), 2-(aralkylamino)-2-thiazolines (AATs), arylethanolamines (AEAs) and 1-(substituted-phenyl)imidazolidine-2-thiones (SPITs) in stimulating adenylate cyclase prepared from thoracic nerve cords of the American cockroach *Periplaneta americana* was examined using parameters calculated by molecular orbital. The hydrophobicity, spatial descriptor and conformational similarity index were the most important factors for adenylate-cyclase activation: the positive solvent-accessible surface (*SAS*) area, log *P* and negative conformational energy by rigid fitting on OA (*CERO*) terms mean that the larger the *SAS*, the more hydrophobic and the smaller *CERO*, the greater the activity in terms of adenylate-cyclase activation.

Keywords: octopaminergic agonist; octopaminergic antagonist; adenylate cyclase; *Periplaneta americana; Locusta migratoria;* molecular modeling; quantitative structure–activity relationship; binding

1 INTRODUCTION

In our recent work,[1–3] octopaminergic-agonist activity was found in 2-(arylimino)thiazolidines (AITs), 2-(aralkylamino)-2-thiazolines (AATs), arylethanolamines (AEAs) and 1-(substituted-phenyl) imidazolidine-2-thiones (SPITs) for stimulating the adenylate-cyclase activity in the thoracic nerve cord of *Periplaneta americana* L. The structure–activity relationships for these compounds were studied in detail.[4–8] It was found that the effect of the ring heteroatoms as well as that of substituents on the phenyl ring was important for the activity of octopaminergic agonists.[2] Some 2-(arylimino)oxazolidines (AIOs) and 2-(aralkylamino)-2-oxazolines (AAOs) were found to be very active in stimulating the adenylate-cyclase activity.[2,4,5,9–13] 2-(4-Chloro-2-methylphenylimino)oxazolidine (AC-6) was a much better acaricide than its thiazolidine and imidazolidine analogs, which corresponded with the potency in activating adenylate cyclase prepared from thoracic ventral nerve cords of *P. americana*.[2] Three-dimensional pharmacophore hypotheses were build from a set of antagonists in the locust nervous system.[14] However, the structural requirements of octopaminergic agonists and antagonists remain poorly understood and hence the present work attempts to establish a better understanding of such quantitative structure–activity relationships (QSAR).

2 MATERIALS AND METHODS

2.1 Chemicals

Octopamine [OA, 2-amino-1-(4-hydroxyphenyl) ethanol], theophylline (1,3-dimethylxanthine) and ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA) were purchased from Nacalai Tesque (Kyoto, Japan); GTP was from

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Sigma Chemical Co (St. Louis, MO); ATP disodium salt was from Kohjin Co (Tokyo, Japan); lithium aluminium hydride (LAH) was from Chemetall GmbH (Frankfurt, Germany).

2.1.1 Radiochemicals

The cAMP radioimmunoassay (RIA) kit (cord RPA 509) was purchased from Amersham International (Buckinghamshire, England).

2.1.2 Synthesis of test compounds

The SPIT compounds were synthesized by the cyclization of monoethanolamine hydrogen sulfate with arylisothiocyanates in the presence of sodium hydroxide as described in a previous report.[11] AIOs and AAOs were obtained by cyclodesulfurizing the corresponding thiourea with yellow mercuric oxide. AITs and AATs were synthesized by cyclization of the corresponding thiourea with concentrated hydrochloric acid or from 2-methylthio-2-thiazoline and corresponding amines. AMT was obtained from 2-mercaptothiazoline and benzyl chloride (**44**) or 4-chlorophenacyl bromide (**45**). AEA (**38**) was obtained by reducing mandelic acid via its ester and amide with LAH and other AEAs were synthesized from trimethylsilyl cyanide and the corresponding aldehyde in the presence of catalytic amount of anhydrous sinc iodide, followed by reduction with LAH, or prepared from hexamethylenetetramine and the corresponding substituted phenacyl bromide, followed by reduction with sodium borohydride. AEA (**85**) was obtained from sodium bisulfite, potassium cyanide and the corresponding aldehydes, followed by reduction with LAH. The structures of the compounds were confirmed by [^{1}H] and [^{13}C] NMR measured with a JEOL JNM-EX400 spectrometer at 400 MHz, tetramethyl silane (TMS) being used as an internal standard for [^{1}H] NMR, and by elemental analytical data.

2.2 Insects

The adenylate-cyclase assay was conducted on adult American cockroaches (*P. americana*) as shown in previous reports.[5,7] Males and females were used indiscriminately, as their nervous systems exhibited no gross structural or neurochemical differences. The insects were reared under crowded conditions in this laboratory at 28°C with a photoperiod of 12 h light : 12 h dark and at a relative humidity of 65–70% for more than seven years; they were provided with an artificial mouse diet (Oriental Yeast Co, Chiba, Japan) and water *ad libitum*.

2.3 Adenylate-cyclase assay

Thoracic nerve cords of *P. americana* were homogenized (15 mg ml^{-1}) in Tris-maleate buffer (6 mM; pH 7.4) by using a chilled microtube homogenizer (S-203, IkedaSci Tokyo, Japan) as shown in previous reports.[5,7] The homogenate was diluted (1 mg ml^{-1}) in Tris-maleate (6 mM), and then centrifuged at 120 000*g* and 4°C for 20 min. The supernatant was discarded, the pellet being resuspended by homogenizing (1 mg ml^{-1}) in the buffer, and again centrifuged at 120 000*g* and 4°C for 20 min. The resulting pellet (P2) resuspended in the buffer was equivalent to the starting amount (15 mg ml^{-1}). The adenylate-cyclase activity was measured according to Nathanson's procedure under optimal conditions[1–11,15–19] in a test tube containing Tris-maleate (120 mM; pH 7.4; 200 µl) containing theophylline (15 mM), $MgCl_2$ (12 mM) and EGTA (0.75 mM) with 60 µl of the P2 fraction and 30 µl of each synthesized compound solution in polyethylene glycol. An appropriate solvent control was run in parallel. The enzyme reaction (5 min at 30°C) was initiated by adding 10 µl of a mixture of GTP (3 mM) and ATP (60 mM), stopped by heating at 90°C for 2 min and then centrifuged at 1000*g* for 15 min to remove the insoluble material. The cAMP level in the supernatant was measured by RIA.[1–11,14–17] Protein concentration was determined by the Lowry method,[20] using bovine serum albumin (Sigma, St. Louis, MO) as the standard. Enzyme activity in each assay was corrected using OA as a reference.

2.4 QSAR analysis

For the QSAR analysis, physicochemical and structural descriptors, including similarity indexes, were correlated with biological activity through stepwise multiple regression using QSAR 2.05, a program designed for Hansch-Fujita's QSAR analysis (Tanabe Pharmac Co Ltd, Osaka, Japan) calculated with a Macintosh personal computer system.

2.5 Molecular modeling

Molecules were built using the Tektronix CAChe WorkSystem™ package of programs (Oxford Molecular Group, OR) on an Apple PowerMac 8100/100AV (100 M BiteRAM, OS J-7.5.1). Molecular geometries in cartesian coordinates of (*R*)-OA were obtained from available X-ray crystallographic data (Cambridge database system) and used as one of the starting points for subsequent calculations. Starting from the characteristic average value angles of each structure, it was energy-minimized using Molecular Mechanics (MM2) and MOPAC (PM3) program (version 94) included in the CAChe system (version 3.8) by connecting to a network server IBM RS6000. The geometries of the energy-minimized molecules were checked using the CAChe 3D visualization tools.

Energy-minimized molecules were generated as follows: firstly, a thorough conformational search was carried out within the molecular mechanics technique (MM2 force field) using initially the steepest descent minimization methods followed by the conjugate gradient until the gradient was below 0.001 Kcal mol^{-1} in no more than 5000 steps. Then the unique minimum was fully optimized with MOPAC (PM3 force field). Thus a Non-Linear Least Squares (NLLSQ) gradient minimization

route by Bartel's method was applied for gradient norm, while with the Broyden–Fletcher–Goldfarb–Shanno (BFGS) method, the nearest minimum-energy geometry was located for optimization. A full Mulliken population was checked to analyze the final Restricted Hartree–Fock (RHF) wavefunction. The PRECISE keyword was also used in order to increase the geometric and electronic convergence criteria. In some cases, the GEO-OK keyword was used to prevent the BFGS routine from unexpected termination. Defaults were used in other settings.

3 RESULTS

In order to understand quantitatively the dependence of binding activities on structure of OA agonists against *Locusta migratoria* L, regression analysis was applied to representative compounds listed in Table 1, leading to eqn (1).

$$pKi = 7.367(\pm 0.349) + 0.163(\pm 0.024)DM - 0.432(\pm 0.050)VCII + 0.539(\pm 0.074)\log P \quad (1)$$

where $n = 52$, $r = 0.900$, $s = 0.520$ and $F = 65.600$. The figures in parentheses are 95% confidence intervals and all terms are justified more than 99% by a t-test: n, the number of data points; r, the correlation coefficient; s, the standard deviation; F, the value of the F-test. Fifty-two molecules that employ a single [^{3}H]OA binding criterion were selected from recent publications by Roeder.[21] Their chemical structures and experimental activities are listed in Fig 1 and Table 1. The respective IC_{50} values of the antagonists had been corrected according to the Cheng–Prusof correction [$K_i = IC_{50}/(1 + K/K_d)$] in order to obtain the K_i values. Log P, log of the octanol-water partition coefficient, is an extremely useful number which is used to describe hydrophobicity. Log P was calculated using the atom typing scheme of Ghose *et al.*[22] In this atom-based approach, each atom of the molecule is assigned to a particular class, with additive contributions to the total value of log P. Valance connectivity index (VCI) is a series of numbers designed by 'order' and 'subgraph type'. There are four subgraph types: Path, Cluster, Path/Cluster and Chain. These types emphasize different aspects of atom connectivity within a molecule – the amount of branching. *VCI1:* first-order (bond) valence molecular connective index ChiIV for the chemical sample.[23] *VCI1* is the

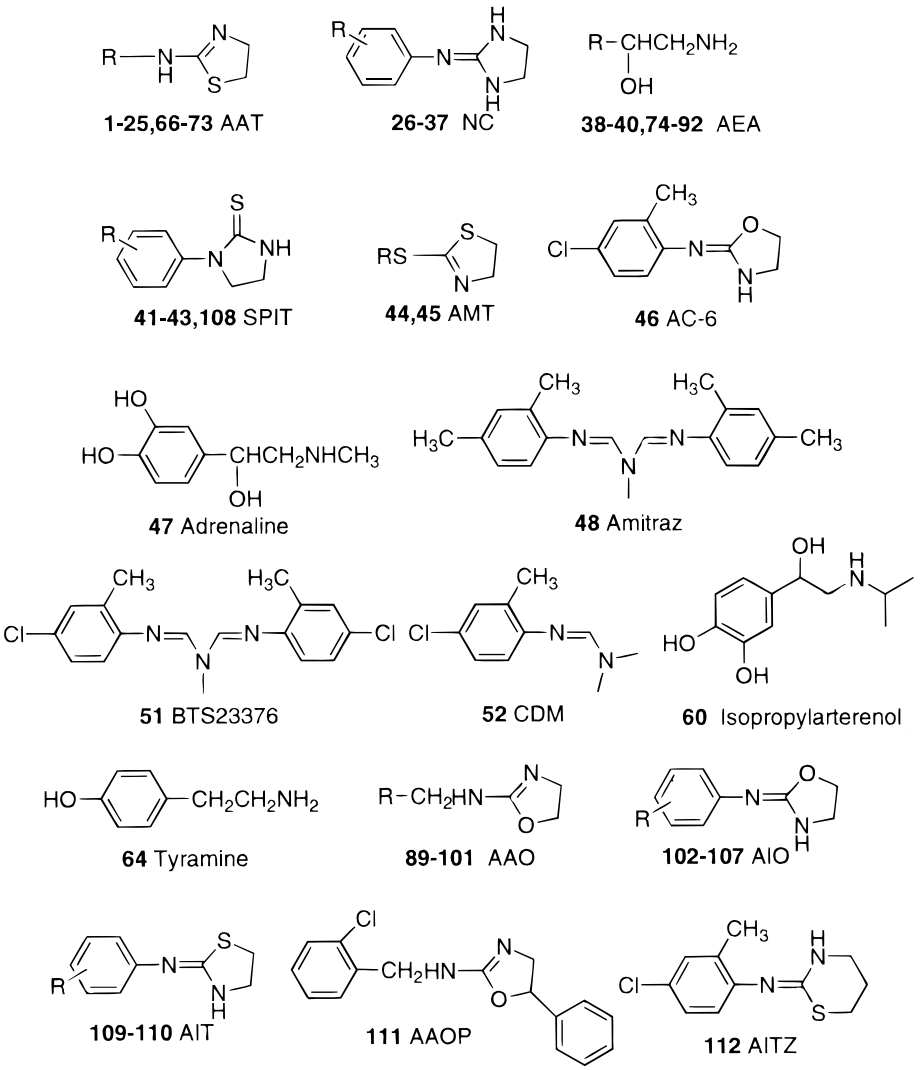

Figure 1. Structures of OA agonists used for regression analysis.

Table 1. Regression analysis of structure-OA agonist and antagonist activities against *Locuta migratoria*

Compound		Ki (nM)	pKi			VCl1	DM	log P
No.	R		Obsd	Calcd[a]	Dev			
1	AAT $PhCH_2$	280 (±59)[b]	6.55	6.47	0.08	5.386	1.047	2.676
2	AAT 2-Cl-$PhCH_2$	440 (±189)[b]	6.36	6.46	−0.10	5.870	0.324	3.194
3	AAT 2-F-$PhCH_2$	447 (±125)[b]	6.35	6.78	−0.43	5.492	2.419	2.815
4	AAT 2-CH_3-$PhCH_2$	650 (±360)[b]	6.19	6.55	−0.36	5.803	0.816	3.143
5	AAT 2-CF_3-$PhCH_2$	290 (±203)[b]	6.54	6.93	−0.39	6.120	2.097	3.559
6	AAT 3-Cl-$PhCH_2$	95 (±66)[b]	7.02	6.54	0.48	5.864	0.731	3.194
7	AAT 3-F-$PhCH_2$	250 (±75)[b]	6.60	6.72	−0.12	5.486	2.098	2.815
8	AAT 3-CH_3-$PhCH_2$	34 (±26)[b]	7.47	6.63	0.84	5.797	1.218	3.143
9	AAT 3-CF_3-$PhCH_2$	380 (±266)[b]	6.42	7.20	−0.78	6.114	3.505	3.559
10	AAT 3-NO_2-$PhCH_2$	185 (±33)[b]	6.73	7.16	−0.43	5.885	5.706	2.629
11	AAT 4-Cl-$PhCH_2$	89 (±6)[b]	7.05	6.57	0.48	5.864	0.885	3.194
12	AAT 4-F-$PhCH_2$	460 (±124)[b]	6.34	6.54	−0.20	5.486	1.190	2.815
13	AAT 4-CH_3-$PhCH_2$	38 (±16)[b]	7.42	6.49	0.93	5.797	0.525	3.143
14	AAT 2,3-Cl_2-$PhCH_2$	42 (±19)[b]	7.38	6.92	0.46	6.353	2.011	3.712
15	AAT 2-Cl,4-F-$PhCH_2$	184 (±90)[b]	6.74	6.71	0.03	5.969	1.368	3.333
16	AAT 2,5-Cl_2-$PhCH_2$	132 (±83)[b]	6.88	6.75	0.13	6.347	1.114	3.712
17	AAT 2,6-Cl_2-$PhCH_2$	1270 (±635)[b]	5.90	6.87	−0.97	6.353	1.749	3.712
18	AAT 3-Cl,4-F-$PhCH_2$	109 (±59)[b]	6.96	6.79	0.17	5.969	1.816	3.333
19	AAT 3,4-F_2-$PhCH_2$	445 (±196)[b]	6.35	6.71	−0.36	5.591	1.810	2.955
20	AAT $PhCH_3CH$ (D)	5000 (±2600)[b]	5.30	5.37	−0.07	9.050	1.010	3.089
21	AAT $PhCH_2CH_2$	5500 (±1920)[b]	5.26	5.33	−0.07	8.887	1.027	2.927
22	AAT 4-Cl-$PhCH_2CH_2$	264 (±95)[b]	6.58	5.37	1.21	9.944	1.624	3.446
23	AAT 3-PyridylCH_2	8330 (±2500)[b]	5.08	5.24	−0.16	8.050	2.549	1.763
24	AAT 1-MorpholinoCH_2CH_2	70 800 (±19 000)[b]	4.15	4.38	−0.23	9.483	1.313	1.600
25	AAT 1-Morpholino$CH_2CH_2CH_2$	121 000 (±31 400)[b]	3.92	4.16	−0.24	10.190	1.376	1.652
26	NC H	23 (±5)[c]	7.63	7.98	−0.35	4.065	9.037	1.808
27	NC 4-Br	15 (±3)[c]	7.83	8.26	−0.43	4.958	9.696	2.599
28	NC 2,4-Cl_2	0.81 (±0.18)[c]	9.09	8.28	0.81	5.026	9.172	2.844
29	NC 2,4-$(CH_3)_2$	1.02 (±0.42)[c]	8.99	8.16	0.83	4.892	8.619	2.742
30	NC 2,6-Cl_2	47 (±18)[c]	7.32	7.15	0.17	5.032	3.401	2.844
31	NC 2,6-$(CH_3)_2$	20 (±7)[c]	7.70	8.26	−0.56	4.898	9.142	2.742
32	NC 2,4,5-Cl_3	2.27 (±0.89)[c]	8.64	8.10	0.54	5.510	7.574	3.362
33	NC 2,4,6-Cl_3	19 (±3)[c]	7.73	8.26	−0.53	5.510	8.397	3.362
34	NC 2,6-Cl_2,4-NH_2	58 (±16)[c]	7.24	6.99	0.25	5.232	5.343	2.060
35	NC 2,4,6-$(CH_3)_3$	4.38 (±1.30)[c]	8.36	8.32	0.04	5.309	8.812	3.209
36	NC 2,4,6-$(CH_2CH_3)_3$	0.56 (±0.14)[c]	9.25	8.41	0.84	6.991	8.789	4.398
37	NC 2,6-$(CH_2CH_3)_2$,4-N_3	1.05 (±0.47)[c]	8.98	9.41	−0.43	6.554	13.144	4.380
38	AEA Ph (DL)	115 (±39)[c]	6.94	6.39	0.55	3.274	2.467	0.791
39	AEA 3-OH-Ph	5050 (±1860)[c]	5.30	6.07	−0.77	3.408	1.951	0.507
40	AEA 3,4-$(OH)_2$-Ph	475 (±42)[c]	6.32	6.40	−0.08	3.549	4.770	0.222
41	SPIT 4-Cl	280 (±134)[b]	6.55	7.02	−0.47	5.417	3.462	2.842
42	SPIT 2-CH_3,4-Cl	20 (±9)[b]	7.70	7.18	0.52	5.834	3.603	3.309
43	SPIT 2,6-$(CH_2CH_3)_2$	170 (±51)[b]	6.77	7.27	−0.50	6.895	3.792	4.051
44	AMT $PhCH_2$	760 (±243)[b]	6.12	5.44	0.68	9.405	2.760	2.866
45	AMT 4-Cl-$PhCOCH_2$	17 500 (±3670)[b]	4.76	4.98	−0.22	10.145	2.942	2.482
46	AC-6	0.95 (±0.24)[c]	9.02	8.67	0.35	4.849	10.469	2.966
47	Adrenaline	416 (±75)[c]	6.38	5.92	0.46	3.994	1.873	0.631
48	Amitraz	22 (±5)[c]	7.67	8.03	−0.36	7.403	3.266	5.809
49	Amitriptyline	1570 (±330)[d]	5.80	6.75	−0.95	7.769	1.326	4.519
50	Antazoline	118 (±62)[d]	6.93	6.88	0.05	7.028	3.047	3.728
51	BTS23376	8.9 (±0.6)[d]	8.05	7.85	0.20	7.537	2.270	5.910
52	CDM	137 (±70)[d]	6.91	7.65	−0.74	4.439	2.847	3.494
53	Chlorpromazine	766 (±54)[d]	6.12	6.38	−0.26	8.331	2.631	3.820
54	Cyproheptadine	844 (±356)[d]	6.07	7.18	−1.11	8.102	0.852	3.926
55	Demethyl mianserine	55 (±3)[d]	7.26	6.30	0.96	7.064	0.408	3.632
56	Desipramine	3210 (±610)[d]	5.49	6.37	−0.88	7.479	1.517	3.645
57	Eresepine	474 (±166)[d]	6.32	5.70	0.62	8.086	0.566	3.208
58	Gramine	1840 (±1012)[d]	5.74	5.89	−0.15	4.469	0.747	1.242

Table 1. *Continued*

Compound		*K*i (*nM*)	*pK*i			*VCII*	*DM*	*log P*
No	*R*		*Obsd*	*Calcd*[a]	*Dev*			
59	Imipramine	1450 (±333)[d]	5.84	6.41	−0.57	7.836	1.281	4.006
60	Isopropylarterenol	18 600 (±4840)[c]	4.77	5.84	−1.07	4.937	0.949	1.386
61	Phentolamine	19 (±9)[d]	7.72	6.58	1.14	7.127	2.772	3.368
62	Promethazine	32 (±23)[d]	7.45	6.39	1.06	7.781	1.687	3.663
63	Triprolidine	2900 (±145)[d]	5.54	6.53	−0.99	7.737	2.631	3.716
64	Tyramine	52 (±18)[c]	7.29	6.47	0.82	3.307	1.899	1.131
65	Yohimbine	82 035 (±22 400)[d]	4.09	4.62	−0.53	9.765	1.738	2.043

[a] Calculated by eqn (2).
[b] Personal communication (T. Roeder, Hamburg University, Germany).
[c] Cited from Ref. 21.
[d] Cited from Ref. 24.

graph edges, that is, the bonds that connect the skeletal atoms, and encodes the number of edges (bonds) in the molecular graph. The number of first-order weights encodes the number of rings. Dipole moment (*DM*, in Debyes) is the magnitude of the molecule's dipole, a 3D electronic descriptor that indicates the strength and orientation behaviour of a molecule in an electrostatic field, which was calculated by MOPAC using PM3 at a minimum energy geometry. Both the magnitude and the components of the *DM* are calculated. It is estimated by utilizing partial atomic charge and atomic coordinates.

According to eqn (1), the hydrophobic and electronic nature of the ligands were the most important factors for binding activity: the positive Log *P*, *DM* and negative *VCI1* terms mean that the more hydrophobic, the larger *DM* values and the smaller the *VCI1* values, the greater the binding activity. Log *P* is widely used to correlate and predict biological activity of pharmaceuticals because activity may depend upon absorption and distribution of drug molecules, which in turn is related to their hydrophobicity. Dipole properties have been correlated to long-range ligand-receptor recognition and subsequent binding. Inclusion of OA antagonists into the above analysis gave a similar result with slightly lower correlation, leading to eqn (2).

$$pK\mathrm{i} = 6.635(\pm 0.344) + 0.194(\pm 0.026)DM - 0.365(\pm 0.050)VCII + 0.596(\pm 0.077)\log P \quad (2)$$

where $n = 65$, $r = 0.873$, $s = 0.603$ and $F = 64.984$. Sixty-five molecules that employ a single [^{3}H]OA binding criterion were selected from recent publications by Roeder.[24] Their chemical structures and experimental activities are listed in Figs 1, 2 and Table 1. The substituent parameters used in the calculation for binding (*pK*i) assay are also shown in Table 1. The introduction of Cl (3), CH_3 (3), NO_2 (3), Cl (4), CH_3 (4), Cl_2 (2, 3), Cl_2 (2, 5) and Cl (3) and F (4) to the unsubstituted AAT **1** increased the potency, leading to **6, 8, 10, 11, 13, 14, 16** and **18,** respectively. Meanwhile, the introduction of Cl, F and CH_3 at *o*-position, F and CF_3 at *m*-position, F at *p*-position, F at *o*- and *p*-positions, Cl_2 at *o*-positions and F at *m*- and *p*-positions, did not have favorable effects, leading to **2–5, 7, 9, 12, 15, 17** and **19,** respectively. Elongation of the alkyl chain between the phenyl and nitrogen of **1** and **2** by a methylene decreased the binding activity to give **21** and **22,** respectively. Heterocyclic AATs **23–25** were not potent OA agonists.

The introduction of Cl and CH_3 at *o*- and *p*-positions, Cl_3, trimethyl and triethyl at 2, 4 and 5, and 2,6-diethyl and 4-azido groups to unsubstituted NC **26** increased the potency, leading to **28, 29, 32** and **35–37,** respectively. Meanwhile, the introduction of Br at *p*-position, Cl_2 and $(CH_3)_2$ at *o*-positions, Cl_3 at *o*- and *p*-positions, Cl_2 at *o*-positions and an amino group at *p*-position did not improve the binding activity, leading to **27, 30, 31, 33** and **34,** respectively.

The octopaminergic-agonist activity for stimulating adenylate cyclase in *P. americana* thoracic nerve-cord homogenates by other compounds was examined similarly in terms of K_a (Table 2).

$$pK\mathrm{a} = 6.239(\pm 0.720) + 0.022(\pm 0.005)SAS + 0.152(\pm 0.054)\log P - 4.260(\pm 0.639)CERO \quad (3)$$

where $n = 64$, $r = 0.904$, $s = 0.244$ and $F = 88.873$. Values of *pK*a, the logs of the reciprocals of *K*a, were used as OA-agonist activity index. *K*a is the concentration of OA agonist necessary for half-maximal activation of adenylate cyclase. The *K*a values were calculated with a Macintosh personal computer system, using a loglinear curve fitting method of KaleidaGraph (3.0.2J). Similarity comparison of molecules was made using atom-based rigid fit method or flexible fitting offered by PowerFit 1.0

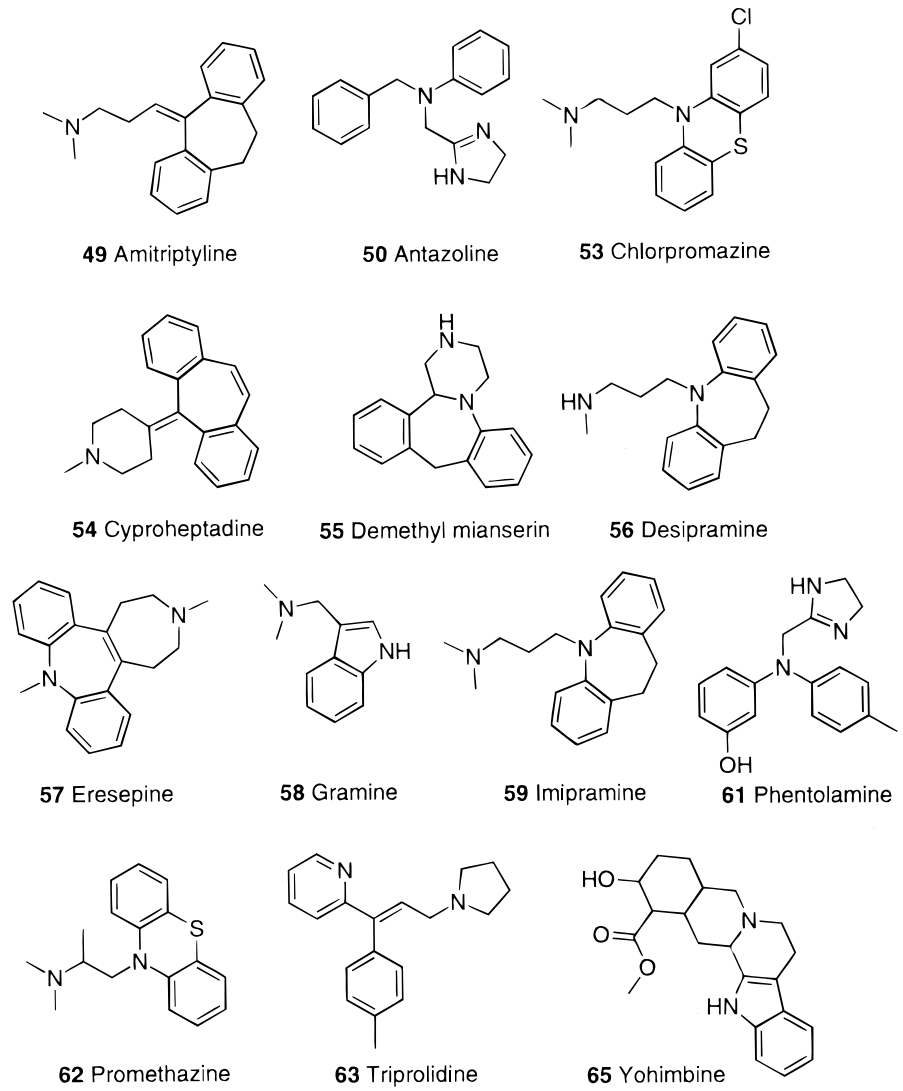

Figure 2. Structures of OA antagonists used for regression analysis.

from MicroSimulations (Mahwah, NJ). PowerFit uses the well-validated Steric and Electrostatic Alignment (SEAL) fitting potential as the objective fitting criterion, and it utilizes the 'global search of best fit' from simulated annealing.[25,26] With this potential, fitting is scored by conformational energy by rigid fitting on OA (*CERO*). Solvent-accessible surface (*SAS*) area, when the solvent is water, is a 3D spatial descriptor that describes the van der Waals area of a molecule. It determines the extent to which a molecule exposes itself to the external environment. This descriptor is related to binding, transport and solubility. According to eqn (3), the hydrophobicity, spatial descriptor and conformational similarity index were the most important factors for adenylate-cyclase activation: the positive *SAS*, log *P*- and negative *CERO* terms mean that the larger the *SAS*, the more hydrophobic and the smaller *CERO*, the greater the activity in terms of adenylate-cyclase activation.

The use of other parameters instead of, or the addition of other parameters to eqns (1)–(3) did not improve the correlation. These included conformation minimum energy (energy calculated for an optimized conformation), CAChe log *P*, which estimates an octanol/water-partition coefficient for any structure which has Molecular Orbital Package (MOPAC) AM1 parameterization for its elements and Log W values estimated by the log octanol-water partition coefficient program (KOWWIN);[27] shape index order 1, 2 and 3 (a topological index quantifying the shape); LUMO energy, the energy gained when an electron is added to the lowest uncoupled orbital; HOMO energy (the energy required to remove an electron from the highest occupied molecular orbital); total energy (the work required to separate the electron and nuclei infinitely far apart) and steric energy (the sum of the molecular mechanics potential energies calculated for the bonds, bond angles, dihedral angles, nonbonded atoms and so forth). The dielectric energy (DE) is a portion of the total energy of a molecule embedded in a dielectric. It is the stabilizing portion that results from screening the charges in the molecule by a dielectric.[28] DE was calculated at an optimized geometry in water. The water geometry was obtained from optimization first

Table 2. Effect of OA agonists on the adenylate-cyclase activity in homogenates of *Periplaneta americana* nerve cords and regression analysis of structure-*pKa*[a]

Compound		Ka (μM)	pKa			SAS	log P	CERO
No	R		Obsd	Calcd[b]	Dev			
1	AAT $PhCH_2$	2.39	5.62	5.38	0.26	104.00	2.676	0.823
66	AAT 2-Br-$PhCH_2$	0.832	6.08	5.87	0.21	117.70	3.468	0.804
3	AAT 2-F-$PhCH_2$	3.67	5.44	5.44	0	105.64	2.815	0.821
4	AAT 2-CH_3-$PhCH_2$	5.21	5.28	5.37	−0.09	109.29	3.143	0.867
5	AAT 2-CF_3-$PhCH_2$	1.38	5.86	5.61	0.25	116.33	3.559	0.861
6	AAT 3-Cl-$PhCH_2$	1.61	5.79	5.85	−0.06	113.96	3.194	0.780
7	AAT 3-F-$PhCH_2$	5.30	5.28	5.55	−0.27	106.45	2.815	0.799
8	AAT 3-CH_3-$PhCH_2$	3.97	5.40	5.50	−0.10	110.76	3.143	0.844
11	AAT 4-Cl-$PhCH_2$	2.39	5.62	5.72	−0.10	114.61	3.194	0.815
12	AAT 4-F-$PhCH_2$	1.69	5.77	5.43	0.34	106.48	2.815	0.828
13	AAT 4-CH_3-$PhCH_2$	5.00	5.30	5.51	−0.21	112.21	3.143	0.850
67	AAT 2,4-Cl_2-$PhCH_2$	1.26	5.90	5.98	−0.08	122.15	3.712	0.809
15	AAT 2-Cl,4-F-$PhCH_2$	3.25	5.49	5.79	−0.30	114.43	3.333	0.802
68	AAT 2-F,4-Cl-$PhCH_2$	1.38	5.86	5.85	0.01	115.88	3.333	0.794
69	AAT 2,4-F_2-$PhCH_2$	4.37	5.36	5.52	−0.16	108.62	2.955	0.823
16	AAT 2,5-Cl_2-$PhCH_2$	0.895	6.05	5.99	0.06	122.31	3.712	0.807
70	AAT 2,5-F_2-$PhCH_2$	3.47	5.46	5.49	−0.03	108.73	2.955	0.830
71	AAT 3,4-Cl_2-$PhCH_2$	0.562	6.25	6.09	0.16	123.70	3.712	0.792
18	AAT 3-Cl,4-F-$PhCH_2$	0.813	6.09	5.83	0.26	116.68	3.333	0.803
19	AAT 3,4-F_2-$PhCH_2$	1.82	5.74	5.57	0.17	109.19	2.955	0.814
72	AAT 3,5-Cl_2-$PhCH_2$	1.05	5.98	6.05	−0.07	125.27	3.712	0.810
73	AAT 3,5-F_2-$PhCH_2$	6.76	5.17	5.56	−0.39	108.75	2.955	0.814
38	AEA Ph (D)	33.9	4.47	4.60	−0.13	81.04	0.791	0.821
74	AEA 2-OCH_3-Ph	34.7	4.46	4.41	0.05	89.07	0.256	0.888
75	AEA 3-Cl-Ph	4.79	5.32	4.92	0.40	91.50	1.309	0.818
76	AEA 3-CH_3-Ph	33.9	4.47	4.52	−0.05	85.80	1.258	0.891
77	AEA 4-Br-Ph	13.5	4.87	5.01	−0.14	96.45	1.583	0.832
78	AEA 4-Cl-Ph	7.08	5.15	4.84	0.31	91.61	1.309	0.837
79	AEA 4-F-Ph	32.4	4.49	4.60	−0.11	84.02	0.931	0.843
80	AEA 4-CH_2CH_3-Ph	22.4	4.65	4.62	0.03	93.72	1.655	0.913
81	AEA 4-OH (OA)	6.03	5.22	4.48	0.74	85.60	0.507	0.864
82	AEA 4-OCH_3-Ph	51.3	4.29	4.35	−0.06	91.27	0.256	0.913
83	AEA 4-SCH_3-Ph	30.9	4.51	4.40	0.11	96.45	−0.486	0.901
84	AEA 4-NO_2-Ph	29.5	4.53	4.52	0.01	92.83	0.745	0.898
85	AEA 2,4-Cl_2-Ph	6.31	5.20	5.11	0.09	100.04	1.827	0.836
86	AEA 2,4-F_2-Ph	18.6	4.73	4.67	0.06	86.59	1.070	0.843
87	AEA 2,4-$(CH_3)_2$-Ph	69.2	4.16	4.48	−0.32	92.42	1.726	0.942
88	AEA 2,5-F_2-Ph	22.4	4.65	4.67	−0.02	86.49	1.070	0.844
89	AEA 2,6-F_2-Ph	34.7	4.46	4.61	−0.15	85.43	1.070	0.841
90	AEA 2,6-$(OCH_3)_2$-Ph	107	3.97	4.40	−0.43	97.89	−0.279	0.915
91	Aea 3,4-Cl_2-Ph	5.50	5.26	5.13	0.13	100.41	1.827	0.832
92	AEA 2-Thiophene	26.3	4.58	4.85	−0.27	79.43	−0.054	0.725
93	AAO cHexyl	13.5	4.87	4.62	0.25	97.21	2.455	0.959
94	AAO 2-Cl-Ph	3.02	5.52	5.40	0.12	106.28	2.851	0.835
95	AAO 3-CF_3-Ph	2.51	5.60	5.49	0.11	112.62	3.215	0.859
96	AAO 3-NO_2-Ph	2.81	5.55	5.31	0.24	109.63	2.286	0.854
97	AAO 4-Cl-Ph	3.23	5.49	5.52	−0.03	108.77	2.850	0.819
98	AAO 2,4-F_2-Ph	11.7	4.93	5.31	−0.38	102.48	2.611	0.829
99	AAO 3,4-F_2-Ph	6.61	5.18	5.28	−0.10	103.17	2.611	0.840
100	AAO 3,5-Cl_2-Ph	1.26	5.90	5.72	0.18	118.74	3.368	0.842
101	AAO 3,5-F_2-Ph	12.0	4.92	5.25	−0.33	103.53	2.611	0.849
102	AIO 4-I	2.57	5.59	5.75	−0.16	114.57	3.238	0.808
103	AIO 2,4-$(OCH_3)_2$	6.31	5.20	5.26	−0.06	110.82	1.476	0.841
104	AIO 2-OCH_3,5-CH_3	2.82	5.55	5.29	0.26	107.68	0.195	0.845
105	AIO 2-CH_3,6-CH_2CH_3	1.70	5.77	5.29	0.48	127.72	4.053	0.806
106	AIO 2-CH_2CH_3,6-$CH(CH_3)_2$	12.6	4.90	5.35	−0.45	120.43	4.038	0.961
107	AIO 2-CH_3,4-Br	2.40	5.62	5.55	0.07	111.74	3.240	0.842

Table 2. *Continued*

Compound		Ka (μM)	pKa			SAS	log P	CERO
No	R		Obsd	Calcd[b]	Dev.			
42	SPIT 2-CH_3,4-Cl	2.57	5.59	5.36	0.23	111.04	3.608	0.896
43	SPIT 2,6-$(CH_2CH_3)_2$	8.51	5.07	5.27	−0.20	116.69	4.350	0.971
108	SPIT 2,6-$[CH(CH_3)_2]_2$	2.82	5.55	5.48	0.07	124.24	5.011	0.983
109	AIT 2-CH_3,4-Br	4.07	5.39	5.68	−0.29	118.00	3.583	0.855
110	AIT 2-CH_2CH_3,4-Br	1.20	5.92	5.91	0.01	124.93	3.979	0.849
111	AAOP	0.331	6.48	6.16	0.32	140.07	4.698	0.892
112	AITZ	6.27	5.20	5.63	−0.43	113.17	3.309	0.882

[a] See Section 2.3 for experimental details. The basal (control) and OA-stimulated adenylate-cyclase activity values were 105.5(±2.5) and 1043.2(±5.0) pmol cAMP min^{-1} mg^{-1} protein, respectively. *K*a indicates the concentration giving half-maximal stimulation. The *K*a values were calculated with a Macintosh personal-computer system, using KaleidaGraph (3.0.2J).
[b] Calculated by eqn (3).

using augmented MM2, then using MOPAC with PM3 parameters. The heat of formation (HF) is the energy released or used when a molecule is formed from elements in their standard states. HF was determined after optimizing the molecular geometry first using augmented MM2 then using MOPAC with PM3 parameters. PM3 HFs have an average unsigned difference between experimental and observed values of 8.6 kcal mol^{-1} for 763 compounds. Excluding Al, P and S the error is 7.1 kcal mol^{-1}.[29] As the steric parameter, molar refractivity (MR) was used, which was calculated using the atom typing scheme of Ghose *et al*.[23] Electron affinity (EA) is the change in the total energy of a molecule when an electron is added. MW is the molecular weight of the molecule and MM3 is minimized energy using a new molecular mechanics force field.[30]

AAOP **111** had the highest potency (*K*a = 0.331 μM), followed by AAT **71, 66** and **16,** for stimulating, adenylate cyclase prepared from thoracic nerve cords of *P. americana* (Table 2). Introduction of CF_3 (2), Cl (3), F (4), Cl_2 (2, 3), F (2) and Cl (4), Cl_2 (2, 5), Cl_2 (3, 4), Cl (3) and F (4), F_2 (3, 4) and Cl_2 (3, 5) to the unsubstituted AAT **1** increased the potency, leading to **66, 5, 6, 12, 67, 68, 16, 71, 18, 19** and **72,** respectively, although the introduction of Cl to the *p*-position of **1** did not affect the potency. Meanwhile, the introduction of F (2), CH_3 (2), F (3), CH_3 (3), CH_3 (4), Cl (2) and F (4), F_2 (2, 4), F_2 (2, 5) and F_2 (3, 5) decreased the potency, leading to **3, 4, 7, 8, 13, 15, 69, 70** and **73,** respectively. A halogen(s) seem(s) to be a favorable substituent on the phenyl ring of AAT compounds for octopaminergic-agonist activity.

Introduction of a phenyl group at C_5 of the oxazolidine ring of **94** increased the activity, leading to **111.** The deletion of the *p*-OH of OA **81** (*K*a = 6.03 μM) dramatically reduced the potency, leading to **38** (*K*a = 33.9 μM), consistent with the result obtained by Harmar and Horn.[31] However, various compounds such as **1, 3–8, 11–13, 15, 16, 18, 19, 42, 66–72, 75, 91, 94–97, 100, 102, 104, 105** and **107–111** had higher potency than OA. These results showed that ring hydroxylation of the AEA nucleus was not essential for the OA-agonist activity and other groups could be substituted for the OH group without a great loss of potency. The introduction of Cl (3), Br (4), Cl (4), CH_3CH_2 (4), OH (4), Cl_2 (2, 4), F_2 (2, 4), F_2 (2, 5) and Cl_2 (3, 4) to the unsubstituted AEA **38** increased the potency, leading to **75, 77, 78, 80, 81, 85, 86, 88** and **91,** respectively, although the introduction of OCH_3 (2), CH_3 (3) F (4), SCH_3 (4), NO_2 (4), F_2 (2, 6) and 2-thiophene did not affect the potency, leading to **74, 76, 79, 83, 84, 89** and **92,** respectively. Meanwhile, the introduction of OCH_3 (4), $(CH_3)_2$ (2, 4) and $(OCH_3)_2$ (2, 6) decreased the potency, leading to **82, 87** and **90,** respectively. The above data suggest that phenyl ring substitution requirements for AAT and AEA derivatives active as octopaminergic agonists differ substantially from each other.

4 DISCUSSION

QSAR is of fundamental importance in modern chemical research. Structural comparison of molecules is the essential first step in the development of QSAR. Traditionally, such comparisons have been made using 2D chemical drawings and 3D plastic models. Advances in computer graphics and molecular mechanics have led to the development of automated procedures or computer-assisted molecular fitting. PowerFit delivers the benefits of two powerful technologies within one intuitive user interface: SEAL fitting energy and 'global search of best fit' from simulated annealing. The first function of PowerFit is to use 3D molecular graphics to replace the plastic model. The intuitive user interface enables the chemist to move, rotate and change the conformation of one molecule to match as closely as possible another molecule. The fitting process can be guided visually or by the fitting energy calculated by using the SEAL fitting potential. Molecular simi-

larity indexes were calculated using the flexible fitting option. This method permits each molecule to adopt that shape (conformation) which maximizes the similarity to a chosen reference molecule, which has been preset to a reference conformation. SEAL combines the electrostatic and steric factors with a Monte Carlo search procedure to provide an automatic and thorough search of structural alignments. The goals and key points are: (1) There are no preassigned atom–atom alignments specified by the user, therefore counter-intuitive alignments will not be ignored. (2) The method is automatic and exhaustive, therefore possible alignments are not missed. (3) The orientations are scored according to a similarity measure, therefore the alignments can be ranked objectively. The surface of the molecular fitting potential has many local minima. In order to find the best fit, an efficient method for the global search of the surface is required. A powerful and well-tested global optimization method, Monte Carlo Simulated Annealing, has been adapted for this purpose.[32] For the fitting of conformationally flexible molecules, selected bonds are rotated to explore the conformational space to find the conformation that best fits the target molecule. In rigid geometry approximation, conformations are generated by rotating about rotatable bonds while the bond length and bond angles are kept fixed. The automated fitting process can be partially or completely directed by chemical intuition in the form of distance constraints. If a pair of atoms should be overlaid, based on chemical intuition, a distance constraint of 0.0 as the targeted value can be imposed.

It was found that conformational indices gave reasonable regression exemplified by eqn (3) in adenylate-cyclase assay. However, in binding assay no significant equations were obtained using similarity index, possibly due to structural variance of test compounds, and *DM* was an important parameter instead, which has been correlated to long-range ligand-receptor recognition and subsequent binding. OA is not likely to penetrate either the cuticle or the central nervous system of insects effectively, since it is fully ionized at physiological pH. Derivatization of the polar groups would be one possible solution to this problem in trying to develop potential OA ligands. Based on the comparison with OA, it was proposed that new compounds could be synthesized which possess similar structural features to OA. Taken together, the above binding and adenylate-cyclase activation studies show that OA agonists and antagonists with certain structural features can be potent ligands of OA receptors. They may help to point the way towards developing extremely potent and relatively specific octopaminergic ligands.

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